DIMENSIONAL AND STRUCTURAL CHANGES DURING DENATURATION OF HELICAL TYPE MACROMOLECULES

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ABSTRACT A theory for quantitative description of reversible helix-coil type phase transition in macromolecular systems is given. The transition is characterized by (1) the fraction of helical content, (2) sequence length of the crystalline regions, (3) sequence length of the amorphous regions, and (4) mean square end-to-end distance. The results for per cent helical or crystalline content are compared with experimental values for polybenzyl glutamate and oligoadenylic acid.

INTRODUCTION

Certain macromolecules such as synthetic polypeptides, proteins, synthetic polynucleotides and deoxyribonucleic acid (DNA) and ribo-nucleic acid (RNA) undergo a reversible phase transition as the temperature is raised. For the natural proteins and nucleic acids, this diffuse phase transition has frequently been termed denaturation. For isolated synthetic linear polypeptides in solution this phase change has been identified as a transition from a helical (crystalline) state to a randomly coiled amorphous state, and has been denoted as the helix-coil transition (Doty *et al*, 1954-57). This transition occurs for single strand helical macromolecules and also for multiple strand helical macromolecules.

The purpose of this paper is to give a quantitative description of this transition. In particular we wish to characterize the macromolecule through the region of transition by the following parameters: (a) per cent helical or crystalline content, (b) sequence length of the crystalline regions, (c) sequence length of the amorphous regions, and (d) mean square end-to-end distance of the macromolecules.

To achieve these purposes we utilize two theoretical developments. The first is a statistical thermodynamic treatment of the phase transition. The second is a theory of chain dimensions of crystalline macromolecules.

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STATISTICAL THERMODYNAMIC TREATMENT. ZIMM-BRAGG THEORY

The prototype theory for the phase transition for linear polypeptides is the theory of Zimm and Bragg (1958). The authors considered the sequence of amide residues in a linear polypeptide chain. The oxygen atom of any given amide residue is either hydrogen-bonded to the hydrogen atom of the third preceding residue or it is not. The first three amide residues are considered unbonded. The notation zero is assigned to an unbonded amide residue (segment) and the notation unity is assigned to a bonded residue. A particular configuration of the chain therefore, would be

$$0\,0\,0\,1\,1\,1\,0\,0\,0\,0\,1\,1.\ldots. \qquad \ldots \quad (1)$$

The following statistical weights were assigned to the various residue pairs

(1) the quantity s for the residue pair 1 1,
(2) the quantity σs for the residue pair 0 1,
(3) the quantity 1 for the residue pair 0 0,
(4) the quantity 1 for the residue pair 1 0

The above assumptions correspond to the simplest form of the Zimm-Bragg treatment. σ is a quantity much smaller than unity and expresses the difficulty of the transition from a non-bonded segment to a bonded segment in a sequence such as (1). The quantity s which is larger than unity when the helix is favoured expresses the tendency for bonded segments to follow bonded segments.

Zimm and Bragg used the matrix method to derive the partition function for the polypeptide chain and an expression for the fraction of bonded segments as a function of s and σ. Their matrix can be expressed as follows, (we have exchanged rows with columns in their notation for consistency with our further discussion)

$$M = \begin{vmatrix} \overline{0\to 1} & \overline{0\to 1} \\ 1\to 0 & 1\to 1 \end{vmatrix} = \begin{vmatrix} 1 & \sigma s \\ 1 & s \end{vmatrix} \qquad \ldots \quad (2)$$

The partition function for a chain of n segments is related to the maximum root λ_{max} of matrix M:

$$Q = \lambda^n_{max} \qquad \ldots \quad (3)$$

where

$$_{max} = \frac{1}{2}\ \{1+s+[(1-s)^2+4\sigma s]^{\frac{1}{2}}\}. \qquad \ldots \quad (4)$$

The fraction of bonded segments θ is equal to

$$\theta = \frac{d\ln\lambda_{max}}{d\ln s}\ . \qquad \ldots \quad (5)$$

A GENERALIZATION OF THE STATISTICAL THERMODYNAMIC TREATMENT

A modification and generalization of the Zimm-Bragg treatment has been presented by Tobolsky (In Press). One reinterprets the partially helical macromolecule as being a sequence of segments which are either in amorphous regions (randomly coiled) and denoted as r or as segments which are in crystalline (helical) regions and denoted as h. A macromolecule can be written, therefore, as

$$r\,r\,r\,h\,h\,h\,h\,h\,r\,r\,h\,h\,h\,r\,r\ldots \tag{6}$$

The question of hydrogen bonded versus non-hydrogen bonded residues is only a special case for the above way of conceptualizing the chain. For a single strand helical macromolecule the crystallization has to be intramolecular sometimes aided by hydrogen bonding but not necessarily so. For a double or multiple strand helical macromolecule, the crystallization will have to be at least partially intermolecular. We may even imagine that the sequence (6) refers to a macromolecule which is part of a semi-crystalline macromolecular system as in bulk poly-ethylene. Here the crystallization is mainly intermolecular.

We also generalize the notation to develop a 2×2 matrix, analogous to the Zimm and Bragg matrix.

(1) a segment pair $r\,r$ is assigned the segment pair partition function f_{rr},

(2) a segment pair $r\,h$ is assigned the segment pair partition function f_{rh},

(3) a segment pair $h\,r$ is assigned the partition function f_{hr},

(4) a segment pair hh is assigned the partition function f_{hh}.

The matrix corresponding to (6) can be written as

$$M = \begin{vmatrix} r\to r & r\to h \\ h\to r & h\to h \end{vmatrix} = \begin{vmatrix} f_{rr} & f_{rh} \\ f_{hr} & f_{hh} \end{vmatrix} \qquad \ldots \quad (7)$$

The mathematical treatment is identical with the Zimm and Bragg treatment if the following identification is made:

$$\begin{aligned} f_{rr} &= 1 \\ f_{hh} &= s \\ f_{rh}f_{hr} &= \sigma s \end{aligned} \tag{8}$$

One can utilize this matrix to develop expressions for the average sequence size in the crystalline regions, the average sequence size in the amorphous regions, etc.

It is very helpful, however, to solve the problem in an alternate manner through the use of segment partition functions rather than segment pair partition

functions. This requires a rewriting of the sequence (6) and redesignating the first segment of every crystalline sequence as k,

$$r\ r\ r\ k\ h\ h\ h\ h\ r\ r\ k\ h\ h\ r\ldots \qquad \ldots (9)$$

The segment partition functions are taken as f_r, f_k and f_h. The solution of the problem now involves the use of a 3×3 matrix as shown by Tobolsky (1962)

$$M = \begin{array}{ccc} \overline{r\to r} & r\to\overline{k} & r\to\overline{h} \\ k\to r & k\to k & k\to h \\ h\to r & h\to k & h\to h \end{array} = \begin{vmatrix} f_r^{\frac{1}{2}}f_r^{\frac{1}{2}} & f_r^{\frac{1}{2}}f_k^{\frac{1}{2}} & 0 \\ f_k^{\frac{1}{2}}f_r^{\frac{1}{2}} & 0 & f_k^{\frac{1}{2}}f_h^{\frac{1}{2}} \\ f_h^{\frac{1}{2}}f_r^{\frac{1}{2}} & 0 & f_h^{\frac{1}{2}}f_h^{\frac{1}{2}} \end{vmatrix} \qquad \ldots (10)$$

The solutions of this matrix are equivalent to the solutions of matrix (2) if :

$$\begin{aligned} f_r &= 1 \\ f_h &= s \\ \frac{f_k}{f_r} &= \sigma s \end{aligned} \qquad \ldots (11)$$

At this point the advantage of this formulation appears. Inasmuch as we use segment partition functions, we can easily interpret these quantities in the following manner :

$$\begin{aligned} f_r &= g_r \\ f_h &= g_h \exp\left(\frac{\Delta H_f}{RT}\right) \\ f_k &= \epsilon \end{aligned} \qquad \ldots (12)$$

In equation (12) g_r is the statistical weight of an r segment, g_h is the statistical weight of an h segment, and ΔH_f is the heat of fusion from the crystalline to the amorphous state. The partition function f_k of the k segments which represent the boundary between amorphous and crystalline regions is taken to be temperature independent (an approximation) and very small, to represent the difficulty of entering such a boundary region.

The following results ensue from equations (10) and (12)

$$T_f = \frac{\Delta H_f}{R \ln \dfrac{g_r}{g_h}} = \frac{\Delta H_f}{\Delta S_f} \qquad \ldots (13)$$

$$\Delta S_f = R \ln \frac{g_r}{g_h}$$

In equation (13) T_f is the transition temperature and ΔS_f is the entropy of fusion.

The results for fractional crystallinity θ as a function of temperature, for the average sequence length $\bar{h}$ in the crystalline regions and the average sequence length $\bar{r}$ in the amorphous regions, both as functions of temperature, are written below.

$$1-\theta = \frac{1-e^{\frac{\Delta F_f}{RT}}+2\frac{f_k}{f_r}+\sqrt{\left(e^{\frac{\Delta F_f}{RT}}-1\right)^2+4\frac{f_k}{f_r}}}{\left(\sqrt{\left(e^{\frac{\Delta F_f}{RT}}-1\right)^2+4\frac{f_k}{f_r}}\right)\left(1+e^{\frac{\Delta F_f}{RT}}+\sqrt{\left(e^{\frac{\Delta F_f}{RT}}-1\right)^2+4\frac{f_k}{f_r}}\right)}, \quad \ldots (14)$$

$$\bar{r} = \frac{\left(1-e^{\frac{\Delta F_f}{RT}}\right)+\sqrt{\left(e^{\frac{\Delta F_f}{RT}}-1\right)^2+4\frac{f_k}{f_r}}}{2\frac{f_k}{f_r}}+1, \quad \ldots (15)$$

$$\bar{h} = \frac{e^{\frac{\Delta F_f}{RT}}\left[e^{\frac{\Delta F_f}{RT}}-1+\sqrt{\left(e^{\frac{\Delta F_f}{RT}}-1\right)^2+4\frac{f_k}{f_r}}\right]}{2\frac{f_k}{f_r}}+1. \quad \ldots (16)$$

$$\frac{n_{seq}}{n} = \frac{\theta}{\bar{h}} \quad \ldots (17)$$

Here

$$\Delta F_f = \Delta H_f - T\Delta S_f . \quad \ldots (18)$$

The expression for θ is essentially equivalent to that given by Zimm and Bragg. We also present explicit equations for $\bar{h}$ and $\bar{r}$. The quantity $\frac{n_{seq}}{n}$ given in equation (17) is the ratio of the number of crystalline sequences to the total number of segments.

Equations (14)–(16) are exact equations deduced from the formulae of reference (4). Simple approximate equations were given also in (4) but, for the purposes of the exact calculations carried out in the subsequent portions of this paper, it was desirable to use the exact equations and machine computations

CHAIN DIMENSIONS DURING THE PHASE TRANSITION

Tobolsky and Gupta (1962) have developed a theory for the end-to-end dimensions R^2 of semi-crystalline macromolecules in terms of two probability parameters.

When statistical thermodynamic equilibrium obtains, these parameters can be related to the partition functions f_r, f_h, and f_k introduced in the previous section. An extremely simple approximate formula results:

$$R^2 = \left[\frac{\theta}{3} (2\bar{h}+1) + (1-\theta) \right] nl^2_0 . \qquad .. \quad (19)$$

where θ and $\bar{h}$ can be identified with equations (14) and (15).

Equation (19), however, applies only to the simplest case where the unit cell of the helix is such that the completely helical form of the molecule is essentially a rigid rod. In other words, we are not considering here the cases where there is complex coiling within the unit cell, as occurs in globular proteins, nor do we consider here the effects which may be produced by long range folding within the crystallites. Although modifications of equation (19) to encompass these phenomena have been given (Tobolsky, 1962; Tobolsky *et al*, 1962) we feel that it is premature to examine these more complex formulae without more experimental evidence than is now available.

Calculations based on equation (19) are nevertheless valuable because in certain cases the completely helical macromolecules do appear to approximate rigid rods.

APPLICATION TO EXPERIMENTAL DATA

In this paper we apply the theoretical results to three helix-coil transitions.

(1) The helix-coil transition of polybenzyl glutamate in a dichloroacetic acid-ethylene dichloride mixture. This is a *reverse* transition, i.e., the helix is stable at high temperatures. This case has been treated by Zimm, Doty and Iso (1959) using the method of Zimm and Bragg. These authors obtain the expression for θ as a function of T by choosing a proper value of σ and ΔH. We repeat these calculations in our notation and in addition present results for $\bar{h}$, $\bar{r}$, $\frac{n_{seq}}{n}$, and R^2.

(2) Schellman postulated that the heat of hydrogen bonding, stabilizing the helix in linear polypeptides is $\Delta H = -1500$ calories/mole. In our notation this means that $\Delta H_f = 1500$ calories/mole. We compute θ, $\bar{h}$, $\bar{r}$, $\frac{n_{seq}}{n}$, and R^2 for the normal helix-coil transition for this type of polypeptide assuming the same σ, and the same transition temperature as in case (1). (The Zimm, Doty and Iso case)

(3) Prof. J. R. Fresco has made available to us preliminary data of Fresco, Blake and Doty for the helix-coil transition of the double stranded helix of oligoadenylic acid. We compute $\bar{\theta}$, $\bar{r}$, $\bar{h}$, $\frac{n_{seq}}{n}$, and R^2 for $\Delta H_f = -1336$ cal/mole and different values of σ.

(1) *Helix-Coil Transition For Polybenzyl Glutamate*

Zimm, Doty and Iso have explained the transition in polybenzyl glutamate in a dichloroacetic acid-ethylene dichloride mixture using the following values for the quantities that appear in the Zimm-Bragg treatment:

$$\sigma = 2\times10^{-4} = f_h/f_r = c/g_r \quad \text{(in our notation)},$$

$$\frac{d\ln S}{dT} = \frac{\Delta H}{RT^2},\ \Delta H = +890 \text{ cal.} = -\Delta H_f \quad \text{(in our notation)},$$

$$T_f = 11.8°\text{C},\quad \Delta S_f = \Delta H_f/T_f = 3.12 \text{ cal/deg.},$$

$$\Delta F_f = \Delta H_f - T\Delta S_f = -890 + 3.12T_f$$

Using these values in equations (14) (15), (16), (17), and (18) we evaluated $\theta, \bar{r}, h, \frac{n_{seq}}{n}$, and R^2 as functions to temperature, using a Bendix (G-15D) type computer. The results are shown graphically in Figures 1-4. The computations for R^2 are purely hypothetical based on the assumption that the helical form of the polypeptide is a rigid rod. We know that this isn't true in this case, since intrinsic viscosity measurements in this system show remarkably little change through the temperature region of the helix-coil transition.

We would like to emphasize that the reverse transition treated by Zimm, Doty and Iso as an application of the Zimm-Bragg theory in fact introduces some grave conceptual difficulties for any statistical thermodynamic model of the types discussed here. These authors correctly stated that the positive value of ΔH (a negative ΔH_f in our notation) can be explained only by solvent effects. However, a negative ΔH_f also means a negative ΔS_f and it is certainly difficult to conceive of the ordered helical state as having a higher entropy than the randomly coiled state.

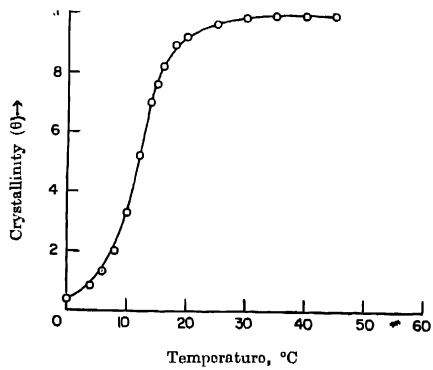

Fig. 1. Crystallinity versus Temperature curve for Polybenzyl Glutamate (Zimm, Doty and Iso case).

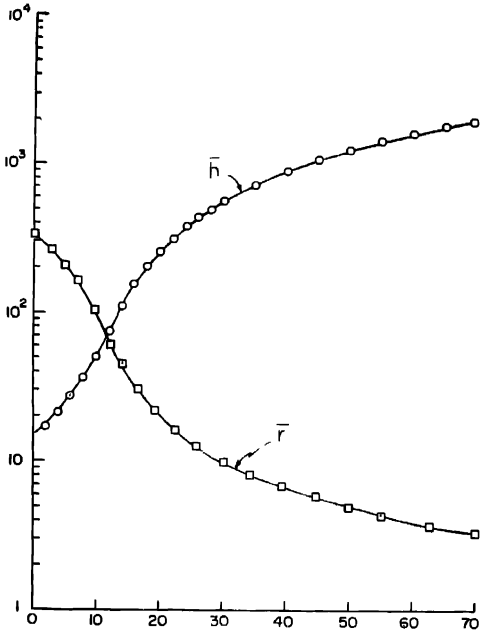

Fig. 2 Curve showing variations of Sequences Lengths in the crystalline and Amorphous Regions with Temperature for Polybenzyl Glutamate

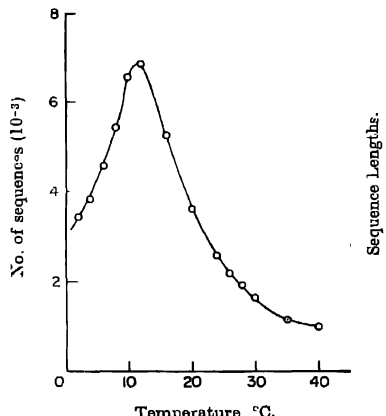

Fig. 3. Number of Sequences Versus Temperature for Polybenzyl Glutamate

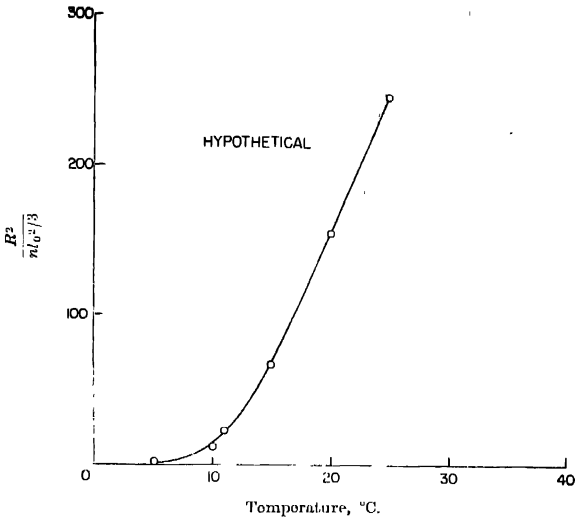

Fig. 4 Variation of the Expected Square of the End-to-End Distance with Temperature for Polybenzyl Glutamate.

(2) *Schellman case*

Schellman (1955) postulated that the heat of dissociation of the hydrogen bond is +1500 cal/mole In other words, the heat of hydrogen bonding stabilizing the helix in linear polypeptides is −1500 cal/mole We use the following values of the various parameters which occur in the expressions for θ, $\bar{r}$, $\bar{h}$, $\frac{n_{seq}}{n}$, and R^2.

ΔH_u (Schellman's notation) $= -\Delta H_f$ (our notation),

$$\Delta H_f = +1500 \text{ cal/mole}$$

For the sake of definiteness in our calculations, we assume that a particular Schellman polypeptide has the same T_f and the same σ as used by Zimm, Doty and Iso.

$$\sigma = 2\times10^{-4} = f_L/f_r = \epsilon/g_r \quad \text{(in our notation)}$$

$$T_f = 11.8°\text{C}, \ \Delta S_f = 5.2668$$

$$\Delta F_f = \Delta H_f - T\Delta S_f = 1500 - T\Delta S_f.$$

The calculations are made for the normal helix-coil transition for this type of polypeptide and the results are shown graphically in Figures 5-8.

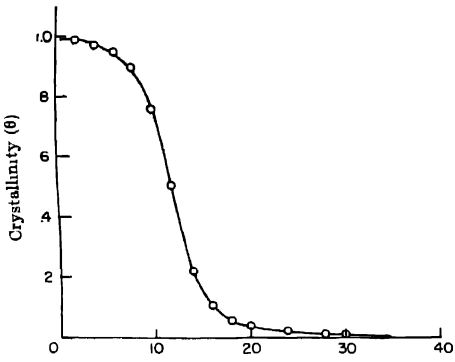

Fig. 5. Crystallinity Versus Temperature Curve for Linear Polypeptides (Schellman Case).

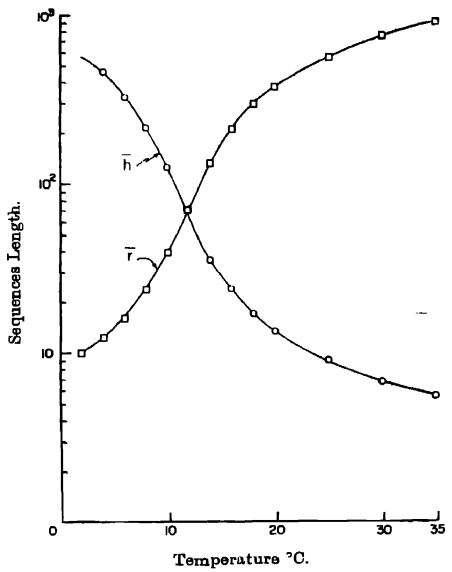

Fig 6. Curve showing Variation of Sequence Lengths in the Crystalline and Amorphous Regions with Temperature for Linear Polypeptides.

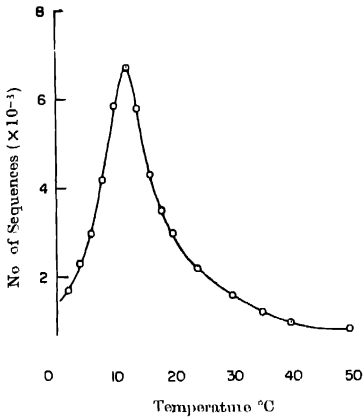

Fig. 7 Number of Sequences Versus Temperature for Linear Polypeptide

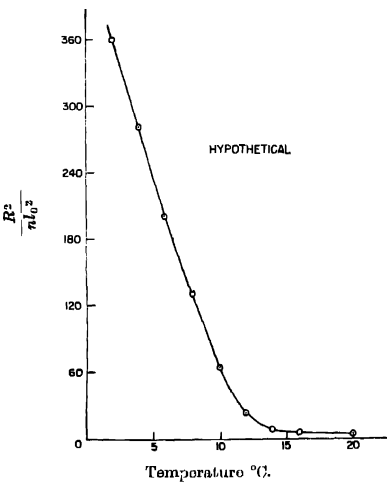

Fig. 8. Variation of the Expected Square of the End-to-End Distance with Temperature for Linear Polypeptides.

(3) *Helix-Coil Transition for Oligoadenylic Acid (Poly A)*

We utilize in this instance some preliminary experimental data on crystallinity versus temperature for a series of oligonucleotides of adenylic acid of vary-

ing degrees of polymerization including data on θ versus T for a very high molecular weight Poly A. The data were kindly supplied to us in advance of publication by Prof. J. R. Fresco (private communication).

The Poly A polymers exist in aqueous solution at low temperatures as double stranded helices. At higher temperatures they undergo a gradual helix-coil transition. The primary experimental data consisted of values for T_f versus the degree of polymerization P. We compute the values of ΔH_f from the formula (Flory, 1957).

$$\frac{1}{T_f} - \frac{1}{T_f^{\infty}} = -\frac{R}{\Delta H_f} \ln\left(1 - \frac{2}{P}\right) \qquad \ldots (20)$$

In equation (20) T_f^{∞} is the melting temperature for the polymer of "infinite" molecular weight and T_f is the melting temperature for the polymer of degree of polymerization P. A plot of $1/T_f$ versus $\ln(1-2/P)$ gave a straight line from whose slope we obtained a value of $\Delta H_f = 1152$ cal/mole. The data in Table I were kindly supplied by Professor J. R. Fresco.

The data for θ versus T for the "infinite" molecular weight poly A were also supplied by Professor Fresco using the optical density method.

The value of $\sigma = f_k/f$, which gave the best fit with the experimental θ versus T data is 1.5×10^{-5}. With this value of σ we then computed θ, $\bar{r}$ and $\bar{h}$. The results are given in Table II.

TABLE I

T_f versus P for Oligo and Polynucleotides of Adenylic Acid in a Sodium Citrate Buffered Solution at pH = 4.0.

P	T_f°C
2	–
3	–
4	—
5	14
6	26
7	—
8	44.5
9	48.5
10	58.5
11	61
infinite	112

TABLE II

Computation of θ, $\bar{r}$ and $\bar{h}$ for Poly A

($f_k/f_l = .000015$)

Temperature C	0 (Experimental)	0 (Theoretical)	r	h
99	1.0	.995	18.5	3800
102.3	.97	.991	26	2500
105	.96	.98	39	1900
106.3	.95	.97	50	1500
108.3	.92	.94	71	860
109.7	.86	.88	100	650
111.0	.75	.73	165	450
111.6	.69	.61	220	340
112.0	.50	.50	260	260
112.4	.23	.40	310	240
112.8	0	.28	400	205

Experimental discussions of the helix coil transition in poly A is given in earlier papers by Fresco and co-workers (1957-59)

A "hypothetical value" for R^2 as a function of temperature could, of course be calculated from Table II and equation (19)

APPENDIX

A Fundamental Critique of the Zimm Bragg Theory

The Zimm Bragg theory has been presented by its authors as a theory for the phase change occurring in linear polypeptides (i.e., the helix-coil transition). It is our contention that this theory is not a true theory of phase transition but rather a *model* for diffuse melting

In order to make our point clear, we first consider a very simple model for the melting of a simple liquid.

Consider a lattice cell model for the liquid state in which all atoms have the partition function $f_L = g_L \exp(E_L/RT)$, consider a lattice model for the solid state in which all atoms have the partition function $f_s = g_s \exp(E_s/RT)$ and consider g_L larger than g_s. Only these two configurations (or microstates) are allowed for the system as a whole.

The partition function of the assembly is

A. 1. $$Q = f_s^N + f_L^N$$

When f_s is larger than f_L, only the first term contributes to Q, when f_L is larger than f_s only the second term contributes to Q. The melting condition occurs when f_s equals f_L. At that point

A. 2. $$\Delta H \text{ fusion} = E_L - E_s$$
$$\Delta S \text{ fusion} = R \ln (g_L/g_s)$$
$$T_m = \Delta H/\Delta S$$

If, on the other hand in our cell model we allowed cells of partition function f_L to mix indiscriminately with cells of partition function f_s the partition function of the assembly would be

A. 3. $$Q = (f_s + f_L)^N$$

Equation (3) shows no phase transition

Although equation (1) definitely shows a phase transition, it was put in there by the assumptions of the model, namely, that there are only two configurations and that the partition functions of the cells are f_L in one configuration and f_s in the other. It is what the old text-books would call a "heuristic proof".

The Zimm-Bragg model when σ equals zero is exactly the same as the model represented by equation (1) The Zimm-Bragg model then gives

A. 4. $$Q = 1^N + S^N$$

We do not regard the Zimm-Bragg treatment as a true theory of phase transition, whether σ is zero or finite.

However, when σ is finite, we regard the Zimm-Bragg treatment as an excellent *model* for diffuse melting. The introduction of finite σ introduces other suitably weighted intermediate configurations in addition to the two displayed in equations (1) and (4). It does not give the same weight to all configurations as was done in equation (3). The mere fact that we assign partition functions 1 and S to segments in random and in helical configurations means that we have preassumed the existence of the two phases The assignment of finite σ means we preassume a boundary phase This is not justifiable if we are considering this treatment as a fundamental theory of phase transition. It is perfectly justifiable if we regard the treatment as a mathematical *model* for diffuse melting.

The very interesting question arises for what problems in diffuse melting can the Zimm-Bragg treatment be applied ? Tobolsky's extension of the treatment, including the generalization of the notation, liberates this model from restriction to the helix coil transition in polypeptides. The effect of the hydrogen bonds, the particular structure of the alpha helix, *perhaps* even the one dimensional aspect of the problem, no longer have central or unique roles.

What systems display diffuse melting ? Isolated polypeptides in solution, globular proteins which denature reversibly, double and triple strand helices such as poly *A*, *DNA*, etc It is perhaps an open question as to whether crystalline polymers in bulk, such as high molecular weight monodisperse polyethylene would show a sharp or diffuse melting under conditions of complete equilibrium. What about selenium, whose crystal structure displays long chains ? Perhaps one requires very thin fibres of polyethylene or thin whiskers of selenium to bring out diffuse one dimensional melting. However, even in low molecular weight systems diffuse meltings are sometimes known to occur. It would be interesting

to know what inherent physical principle underlies diffuse melting (excluding trivial cases such as the effects of impurities). In response to our questionings Professor F. R Eirich made the interesting suggestion that the basic thing that might underlie diffuse melting is anisotropy of the radial distribution function in the liquid state

The basic question is to what extent would a generalized Zimm-Bragg treatment, such as that presented by Tobolsky, be an apt model for diffuse melting in general ? The double strand helix of poly *A* may be a crucial case. If the treatment presented in this paper is a good approximation for poly *A* it might encourage further application of the concept to other cases of diffuse melting.

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